MORPHOLOGICAL ADAPTATION TO THERMAL STRESS IN A MARINE FISH, *FUNDULUS HETEROCLITUS* [1,2]

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Clinal morphological variation of marine fishes has been observed within and across species (Barlow, 1961). This variation is generally associated with differences in the thermal regime and can be due primarily to various developmental responses to environmental cues (Fowler, 1970) or to frequency differences of polymorphic genes. In either case, once latitudinal patterns of variation have been described, temperature stresses for a particular population may be estimated. The effects of a thermal environmental perturbation upon the morphological characteristics of a population of *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and an interpretation of how these effects may be of adaptive significance to natural populations experiencing relatively high temperature are presented in this study.

MATERIALS AND METHODS

Fundulus heteroclitus is a common fish in salt and brackish water from Newfoundland to the Mantanzas River in northern Florida. Latitudinal variation is characterized here with population samples in Long Island Sound, and Ladies Island, South Carolina. Two control populations in Long Island Sound flank a thermal effluent produced by an electric generating plant at Northport, New York, on the north shore of Long Island. One control locality is a large salt marsh approximately fifteen miles east of Northport (Flax Pond), and another is a harbor five miles west of Northport (Centerport). Temperatures during this study were 12° to 15° C higher at Northport than at either flanking control locality, which varied from 0° C in winter to about 20° C in summer. Summer temperatures at Northport approached the thermal tolerance limit of *F. heteroclitus*. Maximum temperatures at Ladies Island, South Carolina, are 3° to 5° C warmer than those in the control localities in Long Island Sound, and the mean temperature at the southern locality is 5° to 7° C higher than the northern control localities. Specimens were collected in traps (cylindrical wire cages with conical indentations at each end) baited with crushed mussels.

For comparison of population samples, the following characters were recorded for each fish: sex, standard length, predorsal length, head length, snout length,

interorbital width, length of dorsal fin insertion, peduncle length, peduncle width, lateral line scales, circumpeduncular scales, predorsal scales, scales above the lateral line, scales below the lateral line, pectoral fin rays, dorsal fin rays, caudal fin rays, and anal fin rays. Morphological measurements followed the criteria used by Hubbs and Lagler (1949), with the following exceptions. Dorsal and anal fin ray counts include all rays that were separated at the base. Caudal fin rays include only the branched rays. Lateral line scales start with the most anterior scale that is fully developed and pored.

The mean standard lengths of specimens from different localities are heterogeneous. In an effort to reduce this heterogeneity, length and width measurements were divided by the standard length of the specimen, and the variable "standard length" was dropped from consideration. Gould (1966, 1971) and Atchley, Gaskins, and Anderson (1976) have shown that this procedure does not necessarily remove all of the size effects and will introduce spurious correlations between standardized variables. The standardization of length and width measurements will remove the size effect for isometric measurements and has been used here for lack of a better technique that may be used upon the available data. The distortion of the covariance structure introduced by standardization is heavily dependent upon the range of relative magnitudes of the coefficients of variation of the numerator and denominator variables (Atchley, et al., 1976). Although the variances of the standard lengths are heteroscedastic, the range of the coefficients of variation (9.6–15.8) is not great. The standardization will alter the correlations

FIGURE 1. An abnormal vertebral column in *F. heteroclitus* (top) contrasted with a normal vertebral column (bottom).

between these variables; however, the concern here is not with the covariance of characters within a population, but with how this structure differs between populations.

X-ray images of skeletons were obtained using a standard medical X-ray machine and Agfa Gaevert industrial film. A focal length of one meter was used with an exposure of 20 seconds, 35 kilovolts, and 50 milliamps, and these settings produced satisfactory images for the full size range of fish measured (40–125 mm). Vertebrae were counted from X-ray images with the aid of a dissecting microscope. A fish was designated abnormal if any of the centra of its vertebral column were fractured, compound, or otherwise grossly misshapen.

RESULTS

Young specimens of *F. heteroclitus* were collected in the spring of 1970 and 1971 from both Northport and Flax Pond. In addition, samples composed of mixed year classes were collected from these localities in the fall of 1971. The number of vertebrae and incidence of vertebral abnormalities were counted from X-ray images of these population samples (Table I). The incidence of vertebral abnormalities was consistently significantly higher at Northport, and in one of the three comparisons, the mean number of vertebrae was significantly lower at Northport. Examples of vertebral abnormalities including foreshortened, asymmetrical, and fractured centra are shown in Figure 1.

Correlations of vertebral number with presence of abnormalities were calculated for three population samples from Northport. In each of the samples, there is a significant negative correlation, indicating that fish with fewer vertebrae had a higher incidence of vertebral abnormalities (Table I). Specimens of *F. heteroclitus* which develop at higher temperatures tend to have fewer vertebrae, and the incidence of vertebral abnormalities in fish with few vertebrae may indicate the limit of this developmental plasticity.

Differentiation of the Northport population with respect to number and form of vertebrae were two of many possible morphological modifications induced by environmental stress. To more accurately assess these modifications, 17 morphological characters were recorded from approximately 30 individuals from the heated locality and each of the control localities (Table II). The extent of the

TABLE I

Analysis of vertebrae in populations of F. heteroclitus. *The correlation between the incidence of vertebral abnormalities and vertebral number is represented by* r.

Collection	Sample size	Per cent with abnormal vertebrae	Mean	s.d.	s.e. of mean	r	P
Flax Pond 1970 Juveniles	69	4.3	33.36	0.66	0.079		
Northport 1970 Juveniles	81	15.8	33.12	0.68	0.075	−0.272	<0.05
Flax Pond 1971 Juveniles	91	2.5	33.19	0.69	0.056		
Northport 1971 Juveniles	153	21.1	33.15	0.46	0.048	−0.220	<0.01
Flax Pond 1971 Mixed	30	3.3	33.27	0.53	0.097		
Northport 1971 Mixed	30	23.3	33.30	0.75	0.136	−0.438	<0.05

TABLE II

Means, sample sizes, and standard errors of morphological variables in F. heteroclitus. *Males and females were coded 1 and 0, respectively.*

Locality (n) Variable	Flax Pond (29) Mean s.e.	Northport (25) Mean s.e.	Centerport (29) Mean s.e.	Long Island (58) Mean s.e.	South Carolina (25) Mean s.e.
Sex	0.414 ± 0.0930	0.490 ± 0.2236	0.483 ± 0.0945	0.448 ± 0.0659	0.360 ± 0.2191
Predorsal length	0.648 ± 0.0038	0.674 ± 0.0112	0.642 ± 0.0050	0.645 ± 0.0032	0.647 ± 0.0085
Head length	0.301 ± 0.0020	0.317 ± 0.0107	0.294 ± 0.0018	0.298 ± 0.0014	0.305 ± 0.0054
Snout length	0.108 ± 0.0013	0.109 ± 0.0045	0.111 ± 0.0011	0.110 ± 0.0008	0.113 ± 0.0036
Interorbital width	0.113 ± 0.0013	0.126 ± 0.0054	0.116 ± 0.0011	0.115 ± 0.0008	0.120 ± 0.0036
Dorsal fin insertion	0.152 ± 0.0030	0.157 ± 0.0063	0.165 ± 0.0029	0.158 ± 0.0022	0.153 ± 0.0076
Peduncle length	0.279 ± 0.0037	0.273 ± 0.0063	0.272 ± 0.0033	0.275 ± 0.0026	0.272 ± 0.0098
Peduncle width	0.165 ± 0.0018	0.178 ± 0.0067	0.170 ± 0.0015	0.168 ± 0.0012	0.165 ± 0.0063
Dorsal fin rays	11.690 ± 0.1226	11.560 ± 0.2911	12.379 ± 0.0917	12.034 ± 0.0885	11.640 ± 0.2853
Caudal fin rays	17.103 ± 0.1876	18.080 ± 0.7424	18.138 ± 0.2841	17.261 ± 0.1825	17.600 ± 0.3873
Anal fin rays	10.586 ± 0.1055	10.600 ± 0.2580	10.897 ± 0.9080	10.741 ± 0.0720	10.520 ± 0.2621
Pectoral fin rays	18.276 ± 0.2098	19.280 ± 0.3770	18.103 ± 0.1812	18.190 ± 0.1379	18.520 ± 0.2281
Predorsal scales	18.586 ± 0.2878	19.120 ± 0.7557	18.379 ± 0.2544	18.483 ± 0.1904	16.600 ± 0.4651
Circumpeduncular scales	19.621 ± 0.1677	20.240 ± 0.5680	20.207 ± 0.1876	19.914 ± 0.1308	19.880 ± 0.3493
Lateral line scales	34.034 ± 0.1448	34.680 ± 0.4226	34.621 ± 0.1818	34.328 ± 0.1215	34.720 ± 0.3542
Scales above lateral line	5.966 ± 0.0345	5.840 ± 0.1673	5.931 ± 0.0479	5.948 ± 0.0293	5.240 ± 0.1950
Scales below lateral line	8.552 ± 0.1536	7.160 ± 0.3578	7.931 ± 0.1484	8.241 ± 0.1136	7.560 ± 0.3672

morphological change in response to the warmer water was analyzed by principal components analysis. The three largest eigenvalues and eigenvectors, or principal axes, of the correlation matrix are presented in Table III. The sum of the first

TABLE III

Eigenvalues and loadings on principal axes from principal components analysis of morphological variation of F. heteroclitus *in a mixed sample from Flax Pond and Centerport (control environments) and Northport (heated environment).*

| Eigenvalues	Principal axes		
	I 3.175	II 2.315	III 1.982
Loadings			
Sex	0.057	−0.411	0.207
Predorsal length	0.352	0.629	−0.233
Head length	0.842	0.094	0.122
Snout length	0.628	−0.221	0.385
Interorbital width	0.880	0.061	0.021
Dorsal fin insertion	0.277	−0.552	0.077
Peduncle length	−0.145	−0.422	0.068
Peduncle width	0.861	−0.149	−0.046
Dorsal fin rays	−0.065	−0.670	0.050
Caudal fin rays	0.158	0.016	−0.341
Anal fin rays	0.120	−0.308	0.114
Pectoral fin rays	0.373	0.076	−0.458
Predorsal scales	−0.228	0.097	−0.613
Circumpenduncular scales	0.124	−0.389	−0.674
Lateral line scales	0.129	−0.188	−0.678
Scales above lateral line	0.001	−0.613	−0.271
Scales below lateral line	−0.255	−0.308	0.069
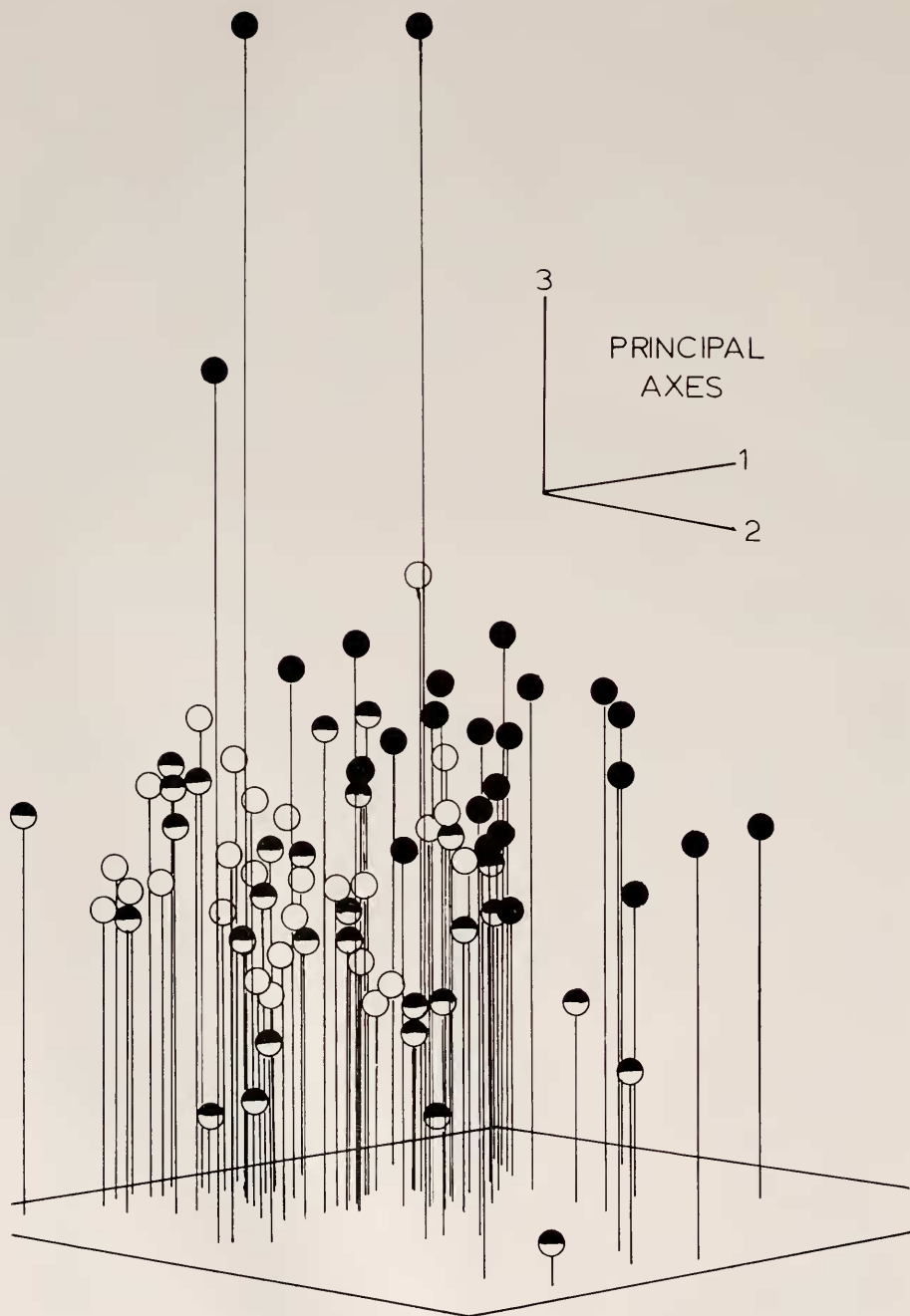
Figure 2. Three-dimensional representation of a principal components analysis of 78 individuals based on 17 morphological characters of *F. heteroclitus* collected from Flax Pond (half-closed circles), Northport (closed circles), and Centerport (open circles) on Long Island, New York in 1971.

three eigenvalues is 8.472, accounting for 49% of the variation in these data. Principal components analyses typically account for a higher proportion of the variance of morphometric variables with three principal axes. The low degree of explanation here seems to be due to the meristic variables, which are essentially independent of one another.

There is a considerable degree of resolution between fish from the warm and control environments. This resolution, seen in a three dimensional space defined by the first three principal axes (Fig. 2) indicates that the Northport population can be distinguished from those of surrounding control environments. Fish from the two control environments are indistinguishable in this analysis.

The characters contributing most heavily to the resolution were identified by discriminant function analysis (Table IV). Head length, snout length, interorbital width, and length of dorsal fin insertion are most important in the differentiation of the warm water population from surrounding natural populations. A frequency distribution of projections of individuals on the discriminant function (Fig. 3) shows Northport individuals are different from individuals collected from either Flax Pond or Centerport.

Morphological differentiation observed at Northport may be a product of different development rates and schedules imposed by the environment and, therefore, only a phenotypic response; or it may be a product of selection for a different phenotype in that environment and actually reflect the evolution of this population. *F. heteroclitus* is known to exhibit considerable morphological variation over its range (Brown, 1957; Relyea, 1967), and has, at times, been broken into races or subspecies, depending upon the investigator. To describe latitudinal variation in this species, a second discriminant function was constructed with both *F. heteroclitus* from naturally cold environments and *F. heteroclitus* from naturally warm environments. Fish from Centerport and Flax Pond were pooled to repre-

TABLE IV

Discriminant functions differentiating the morphology of F. heteroclitus *from several environments.*

	Discriminant function coefficients	
Characters	Heated and control environments Long Island Sound	Northern and southern environments
Sex	2.619	−0.127
Predorsal length	−13.679	−9.168
Head length	−111.470	−12.287
Snout length	−5.682	37.244
Interorbital width	−18.274	−26.096
Dorsal fin insertion	31.927	−9.099
Peduncle length	−8.534	−4.683
Peduncle width	53.051	−2.183
Dorsal fin rays	−0.548	0.062
Caudal fin rays	0.480	−0.105
Anal fin rays	−0.378	0.058
Pectoral fin rays	−0.297	−0.208
Predorsal scales	1.304	−0.091
Circumpeduncular scales	−1.335	−0.161
Lateral line scales	−0.428	0.067
Scales above lateral line	9.064	0.224
Scales below lateral line	0.748	0.343

sent fish from a natural cold environment, while fish collected from Ladies Island, South Carolina (Table II) represented *F. heteroclitus* from a natural warm environment. Characters that differ between warm and cold natural environments are those associated with head shape, as well as scales above the lateral line (Table IV). Although there is some overlap of the northern and southern populations, the means of these samples are significantly different ($F_{17,\ 65} = 8.98$, $P < 0.001$). A frequency distribution of projections of individuals on the discriminant function (Fig. 4) shows that individuals from Northport span the entire range of variation seen in the two natural environments. Again, although there is considerable variation in the morphology of the specimens from Northport, the mean for the Northport population is significantly different from both the control populations in Long Island Sound ($F_{17,\ 65} = 3.05$, $P < 0.001$) and the population from Ladies Island, S. C. ($F_{17,\ 32} = 3.52$, $P < 0.005$). Thus, the population exposed to the industrial thermal effluent is differentiated from surrounding control localities, and shows convergence to phenotypes abundant in naturally warm environments.

Finally, one may wonder about the relative amounts of morphological variation in the natural and stressed environments. The extreme temperatures at Northport are severe enough to produce an increased level of vertebral abnormalities, and these temperatures may lie outside the range in which the genotypes are buffered or exhibit developmental homeostasis, resulting in a greater range of morphological variation. On the other hand, the selection at Northport may have been directional to such an extent that the genetic variation underlying morpho-
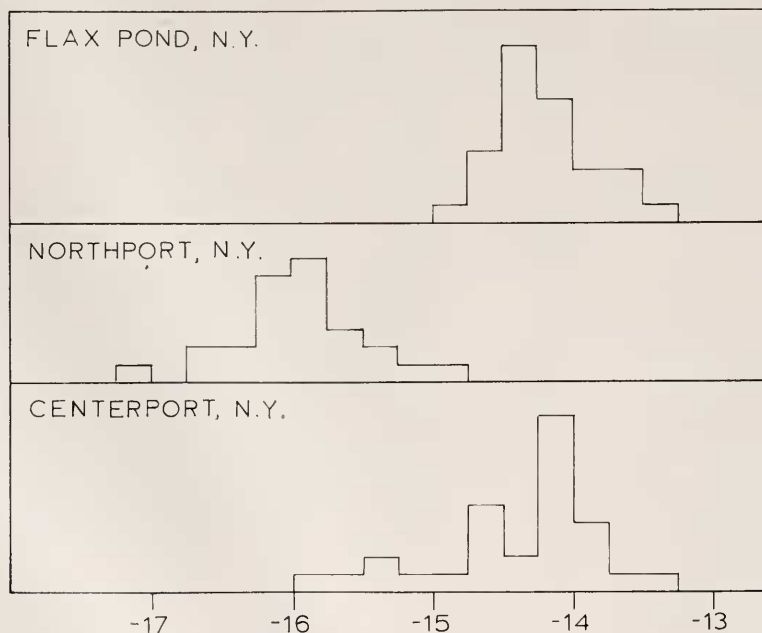

FIGURE 3. Frequency distributions of projections of individuals of *F. heteroclitus* from Flax Pond, Northport, and Centerport upon a discriminant function constructed from variance–covariance matrices of 17 morphological characters of fish from Flax Pond and Northport.
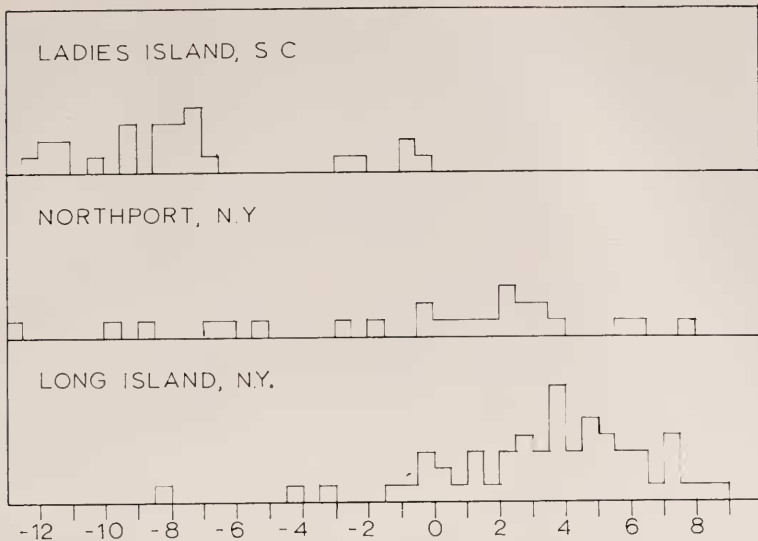
FIGURE 4. Frequency distribution of projections of individual of *F. heteroclitus* from Long Island Sound, New York, Northport, New York, and Ladies Island, South Carolina upon a discriminant function constructed from variance-covariance matrices of 17 morphological characters of fish from Long Island, New York and Ladies Island, South Carolina.

logical variation may have been depleted, leaving a restricted range of morphology. Other hypotheses may also be constructed. Resolution of these questions would ideally lie in comparisons of multivariate variances of populations, so that many characters and the correlations among them may be considered simultaneously. Unfortunately, an ideal measure of multivariate variance is not available. Sokal (1965) has suggested the generalized variance, which is the determinant of the variance-covariance matrix and is proportional to the volume of points in an n-dimensional hyperspace. Soulé (1972), in a search for a population variation parameter, finds fault with the generalized variance and offers instead a mean coefficient of variation. The mean coefficient of variation avoids problems with singular matrices, but ignores correlations between characters. Both measures are employed in this study (Table V).

In order to avoid inflated variances due only to allometric differences in samples with heterogeneous age distributions (Gould, 1966), the morphological variables have been restricted to meristic variables, determined early in development and constant thereafter. The counts employed are scale counts below the lateral line, along the lateral line, around the caudal peduncle, and fin ray counts of the dorsal, caudal, anal, and pectoral fins. Where sample sizes permit, variances are given separately for the sexes but sexual dimorphism for these seven characters is not evident. For both measures of multivariate variance, and in all comparisons, the variance is greater at Northport than in any of the control localities (Table V). The difference is most pronounced when measured by the generalized variance, which utilizes both the variances of the characters and the covariances among them.

Multivariate variances for seven meristic characters of F. heteroclitus *from control and heated (Northport) localities.*

Sample	Sample size	Generalized variance	Mean coefficient of variance
Samples sorted by sex			
Flax Pond males	88	0.080	5.77
Flax Pond females	178	0.056	5.58
Northport males	111	0.202	6.32
Northport females	195	0.136	6.04
Samples including both sexes			
Flax Pond	29	0.024	5.56
Centerport	30	0.023	5.79
Ladies Island, S. C.	25	0.003	5.12
Northport	24	0.052	6.36

DISCUSSION

Gabriel (1944) performed experiments upon subsets of sibships to determine which, if any, factors influence the number of vertebrae in *F. heteroclitus*. The number of vertebrae were influenced by genetic factors, temperature dependent factors, and temperature independent factors. Sibs raised at different temperatures had different numbers of vertebrae, with vertebral number decreasing with increasing temperature of development. Although this result is straightforward, mechanisms underlying temperature dependent shifts in meristic characters of teleosts are complex (Fowler, 1970). Gabriel also found a phenomenon, presumably with a genetic basis, that interfered with predictions of the response of vertebral number to temperature. Some sibs responded to temperature in the manner described above, but in others the response was mitigated. Tåning (1952) found V-shaped rather than linear functions of the mean count of a meristic variable with respect to temperature during development, and it is not implausible that the underlying mechanism may apply to characters with continuous variation as well. Barlow (1961) proposed that temperature dependent shifts in meristic characters are the product of two separate temperature dependent rates, the rate of growth or increase in mass, and the rate of development or differentiation. If one rate is more temperature dependent than the other, different counts will result at different temperatures of development. A V or inverted V-shaped function will result if one of the rates has an inflection point.

Thus, the determination of the state of a meristic variable is complex, and reliable quantitative predictions from knowledge of temperature might be too much to expect. Although Gabriel (1944) found vertebral number to be a linear function of temperature of development, the possibility that other meristic counts and variables with continuous variation might have more complicated response functions to temperature, or no response at all, cannot be ruled out. Therefore, knowledge of processes underlying the determination of states of meristic or continuous characters is not sufficient to allow us to predict the magnitude of a morphological change for a given environmental perturbation, or perhaps even its direction. From Gabriel's work (1944), however, one would predict that vertebral number would be lower in the elevated temperatures at Northport, if

they were different at all. In one of three comparisons (Table I), significantly fewer vertebrae were found in fish from Northport.

A prediction may also be based upon the latitudinal variation in morphology, which is presumably a response to temperature. If the morphological differentiation at Northport is predominantly a response to temperature, the changes occurring there should make that population resemble the morphology of southern populations. Support for this hypothesis is seen in Figure 4.

Evidence that the environment at Northport imposes a stress upon *F. heteroclitus* is provided by the elevated frequency of vertebral abnormalities observed there. A similar observation was made by Hubbs (1959), who found vertebral abnormalities to be common in the mosquito fish, *Gambusia affinis*, living in natural hot springs in Texas and Mexico. Higher incidence of vertebral abnormalities in fish with fewer vertebrae (Table I) suggests that fish marked with a vertebral abnormality are individuals whose tolerance limits had been exceeded, and whose canalization has been disrupted. The fish with severe abnormalities, as well as the individuals that disappeared before the time of sampling, may be carriers of different genes, or different combinations of genes, than those that seemed to have developed normally. If many genes contribute to set the physiological environment of the fish and those genes have pleiotropic effects, the opportunity for genetic and genetically based morphological divergence of this population is great. The incidence of severe vertebral abnormalities marks a clear opportunity for selection to act, and an analysis of enzyme polymorphisms (Mitton, 1973; Mitton and Koehn, 1975) provides evidence that selection has operated. A large gravid female may carry several hundred eggs at one time and may have two spawning periods, each several months in length (Mathews 1938). This high fecundity, and high degree of genetic variability discovered (Mitton 1973; Mitton and Koehn 1975) may permit rapid differentiation in a severe environment.

Specimens of *F. heteroclitus* at Northport generally have longer and wider heads than those of *F. heteroclitus* at surrounding control localities, and at the same time, have more and larger gill filaments (G. Williams, State University of New York at Stony Brook, personal communication), providing them with greater gill surface area. The need for greater gill surface at this locality is clear. Metabolic rates of these fishes are enhanced by higher temperatures, but the amount of oxygen dissolved in the water is likely to be lower. Thus, the larger head dimensions in warm water populations are a reflection of greater gill surface area, which is demanded by the greater need of oxygen combined with its lower availability.

Breeding studies in fishes, including *F. heteroclitus*, have amply demonstrated that morphology has a genetic basis (Gabriel, 1944; Hagen, 1973) and is influenced by the environment. Complex mechanisms underlying morphology (Gabriel, 1944; Tåning, 1952; Barlow, 1961; Fowler, 1970), however, leave little hope of predicting morphological variation in given circumstances or understanding of whether changes observed are strictly developmental phenomena and/or have a substantial genetic component. Discriminant function analysis has shown the morphology of *F. heteroclitus* at Northport to be different than morphology in surrounding localities and has revealed a possible convergence towards the phenotype of southern populations, but there are no direct data that show this phenotypic convergence to have a genetic basis.

A multivariate analysis of both morphological and protein phenotypes performed upon these population samples (Mitton, in preparation) reveals that morphological variation is not independent of protein variation. In addition, analyses of protein variation identified genetic divergence of the artificially-heated population from surrounding control localities (Mitton, 1973; Mitton and Koehn, 1975). If allelic frequencies at polymorphic loci controlling morphological characters have been altered to the same extent as the allelic frequencies of loci coding for enzymatic proteins, much of the morphological differentiation observed here could have a genetic basis.

Several mechanisms may be presented to explain the greater range of morphological variation at Northport than in the control localities; and although none can be firmly rejected, some are more plausible than others. Soulé (1971) and Morris and Kerr (1974) have reported correlations between genic heterozygosity as measured by protein polymorphisms and some measure of morphological variation. Levels of heterozygosity tend to be higher at Northport than at Flax Pond (Mitton and Koehn, 1975), but the difference is slight and is probably not sufficient to explain the two-fold difference in variation detected by the generalized variance (Table V). Fisher (1930) demonstrated that the rate of evolution of a population was proportional to its variance in fitness, and the Northport population is presented as a population adapting to a new environment, but it has not been demonstrated that variation in meristic characters of *Fundulus* is adaptive variation (Tables III, IV, V). Meristic variation has been implicated in selection (Fox, 1975 and references therein), but the data presented here neither suggest nor deny an adaptive function of this variation. Although rigorous selection is generally presented as a force decreasing variation, Warburton (1967) has shown that if rare phenotypes are favored, selection will actually result in an increase in variation. The morphology of northern control localities is different from the morphology typical at the southern control locality (Fig. 4), and the morphology of *F. heteroclitus* at Northport seems to be converging toward the southern morphology, so the situation envisioned by Warburton (1967) seems to be realized at Northport. Although disruption of developmental homeostasis (Table I) may contribute to the variation at Northport, the large amount of morphological variation may also be an adaptive concomitant of selection for an unsual phenotype.

SUMMARY

Populations of *Fundulus heteroclitus* (Cyprinodontidae), a coastal marine fish, were studied in control and artificially heated environments on the north shore of Long Island to determine patterns of variation in morphology and the extent to which this variation reflected adaptation to environmental characteristics.

Principal components and discriminant function analyses were used to analyze variation in and among seventeen morphological characters. Fishes living in water artificially heated by a power plant exhibited marked divergence from control populations in head morphology, and convergence with a population sampled at more southern latitudes. Hence, these differences were interpreted as adaptations to warm environments. Greater morphological variation is detected at the heated locality than at control localities, and this may be partially due to a breakdown in developmental homeostasis, and partially due to selection favoring phenotypes that are rare in this environment.

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